Epstein-Barr virus infection in the neonatal period and in childhood

J. Joncas,*† M.D., J. Boucher,* B.SC., M. Granger-Julien,* B.SC. and C. Filion,* R.N., *Montreal*

Summary: In an attempt to detect and characterize congenital, neonatal and early childhood EBV infections, a prospective sero-epidemiological study was undertaken in 112 newborn infants and their mothers, 25 additional newborns undergoing exchange transfusion, 114 randomly selected hospitalized infants aged 0 to 3 years, and 109 siblings and parents of these infants. Leukocyte culture was attempted in all the newborns and in 25 pre- and post-transfusion.

The findings of EBV seroconversion in six patients without clearly apparent illness, infectious mononucleosis in only one case with significant EBV antibody rise, seroreversion in three cases in early childhood, higher newborn than maternal EBV antibody titres in three cases and the establishment of two permanent lymphoblastoid cell lines from newborns following exchange transfusion raise the possibility of abortive primary EBV infection in early life. Congenital or neonatal infections following exchange transfusions, however, could not be substantiated with certainty since the EBV antibodies did not persist at follow-up except possibly in two cases. Parenteral transmission of the EB virus by exchange transfusion at birth is probably prevented by the presence of EBV antibodies in either donor or recipient.

Résumé: *L'infection à virus Epstein-Barr (VEB) durant la période néonatale et l'enfance*

Dans une tentative de découvrir et de caractériser les infections à VEB, congénitales, néonatales et du début de l'enfance, nous avons entrepris une étude prospective séroépidémiologique chez 112 nouveau-nés et leurs mères, chez 25 autres nouveau-nés qui étaient soumis à des exsanguinotransfusions, chez 114 nourrissons hospitalisés pris au hasard, âgés de 0 à 3 ans, et chez 109 frères, soeurs et parents de ces nourrissons. On a tenté des cultures de leucocytes chez tous les nouveau-nés et chez les 25 nouveau-nés transfusés, tant avant qu'après les transfusions.

La découverte d'une séroconversion de VEB chez six malades exempts de maladie clairement établie, d'une mononucléose infectieuse dans un seul cas, où il y avait une nette augmentation du titre des anticorps de VEB, dans trois cas d'une séroréversion survenue au début de l'enfance, d'un titre d'anticorps de VEB plus élevé chez le nouveau-né que chez la mère dans trois cas, et l'obtention de deux lignées cellulaires lymphoblastoïdes permanentes chez des nourrissons ayant subi des exsanguinotransfusions, toutes ces constatations évoquent la possibilité d'une infection primaire à VEB qui aurait avorté au début de la vie. Toutefois, il ne serait pas possible d'établir avec certitude la présence d'infections congénitales ou néonatales consécutives à l'exsanguinotransfusion étant donné que les anticorps n'avaient pas persisté lors de l'examen de contrôle, sauf peut-être dans deux cas. Du reste, la transmission parentérale du virus EB par l'exsanguinotransfusion à la naissance est probablement évitée par la présence des anticorps de VEB, soit chez le donneur, soit chez le receveur.

Sero-epidemiological studies[1-9] of families, college students, armed forces personnel and other populations have shown EBV seroconversion rates varying from 1.8[4] to 27%[3] per year but there appears to be no significant difference in conversion rates between populations with and without index cases of infectious mononucleosis as a source of infection.[1,3,8] The present study was undertaken in an attempt to detect and characterize congenital, neonatal and early childhood EBV infections. We also wanted to find out if the EBV infection would be more readily transmitted from these infants than from patients with mononucleosis[1,3,5-9] and finally if such a transmission would result in the occurrence of infectious mononucleosis in older children and young adults in the same family.

Material and methods

Heparinized blood was collected from a group of 25 newborns prior to and following exchange transfusion and leukocytes cultures were started according to the technique of Moore, Gerner and Minowada.[10] Blood used for the transfusion had been freshly collected within 48 hours. Epstein-Barr virus antibody titres in the serum of these newborns and their mothers, at birth and at intervals of three months thereafter, were determined by the Henles's indirect immunofluorescence test.[11]

*Institute of Microbiology and Hygiene of Montreal
†Departments of Microbiology and Pediatrics, University of Montreal and Sainte-Justine Hospital, Montreal
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Reprint requests to: Dr. J. Joncas, Institut de microbiologie et d'hygiène de Montréal, C.P. 100, Ville de Laval, Qué.

An additional group of serum samples taken at birth from 112 infants and their mothers were also screened for EBV antibodies and the leukocytes of these newborns were put into culture. Another group of 265 serum samples from 114 infants admitted to Sainte-Justine Hospital for various reasons and subsequently followed up at three- to six-month intervals were also tested for the same antibodies. Finally 147 serum samples from 109 family contacts of the latter group of 114 infants were similarly tested for EBV antibodies. Serum from the EBV seroconvertors was submitted to the Monospot test (Ortho Diagnostic).

Cells of the HRIK clone of the P3J Burkitt lymphoma cell line were used as a source of antigen-positive cells and indirect immunofluorescence was performed with serum in twofold dilutions from 1/5 to 1/320.[1,6] Complement fixation tests for EBV antibodies were kindly performed by Dr. Paul Gerber* on selected sera from 11 infants, 3 to 20 months following exchange transfusion. EBV antibody titres mentioned in the text or appearing in the table are titres obtained by immunofluorescence except when otherwise indicated.

The presence of EB virus antigen-positive cells in established cell lines from the newborns was determined after the examination of at least 300 cells stained by indirect immunofluorescence with known positive and negative sera. Cell suspension samples from the same cell lines were examined for the presence of herpesvirus particles. Following three cycles of freezing and thawing they were centrifuged at 80,000 G. for two hours. This high-speed pellet from the original cell suspension was resuspended in a ratio of 20/1 in RPMI 1640 medium. A 0.01 ml. quantity of the sample was placed on a carbon-coated grid resting on an agar plate. After a 90-minute contact the grid was dried completely with filter paper and a 3% solution of phosphotungstic acid, pH 6.0, was added for negative staining of the virus particles. It was examined with a Philips 200 electron microscope. Karyotype analysis of established cell lines was done following the method of Moorhead *et al.*[12]

Results

In the group of newborns two permanent lymphoblastoid cell lines were obtained (Table I). The first lymphoblastoid cell line with a female karyotype was EBV-positive by immunofluorescence and electron microscopy (Fig. 1). It was obtained from the post-exchange blood sample of a female newborn infant (Ia) who had received a transfusion four weeks before birth from a male donor with an EBV antibody titre of 1/160. Another exchange transfusion was given at birth, also from a male donor with an EBV antibody titre of 1/40. Permanent lymphoblastoid cell lines could not be established from the leukocytes of donors or mother. The cell cultures from one of the two donors and from the mother, however, proliferated with cell clumps and were metabolically active for six weeks. The EBV antibody titre of the infant was 1/20 prior to the exchange transfusion and 1/40 following the transfusion. The EBV antibody titre of the mother was 1/40 when the baby was three months of age. EBV antibodies were not detected in the serum of the infant at 3 and 9 months except in low titre by complement fixation (Table I).

The second lymphoblastoid cell line derived from a male newborn (Lep) infant was EBV-positive by immunofluorescence only. It was also obtained from the post-exchange blood sample following transfusion from a male donor. The EBV antibody titre of the infant was 1/80 prior to the exchange transfusion and 1/40 following the transfusion. EBV antibodies were not detected in the serum of the infant at 6 months except in low titre by complement fixation. In at least one baby (Pa) EBV antibodies persisted at 6 and 9 months at a titre of 1/5, equal to that found at birth. Another baby (Bl) who could not be tested at 3 and 6 months was found to have at the age of 9 months the same EBV antibody titre as at birth, 1/40. It was 1/5 at the age of 12 months. By complement fixation test, EBV antibodies could be detected in all but two (Du and Ho) of all 11 infants tested. Total serum IgM in the cord blood of four babies, Ia, Lep, Pa and Bl, was measured by the Hyland immunoplate test. It was not found to be abnormally elevated (13 to 23 mg./100 ml.).

A further group of 112 paired serum samples taken at birth from newborn infants and their mothers was also screened for EBV antibodies. In 3 of the 112 pairs the infant's titre was higher than the mother's titre by a twofold dilution, and in 21 of the 112 pairs by a single-fold dilution. In 57 of the 112 pairs the mother's titre was equal to the infant's titre and ranged from 1/5 to 1/320: 1/5 (2), 1/10 (4), 1/20 (6), 1/40 (15), 1/80 (13), 1/160 (15), 1/320 (2). In 12 of the 112 pairs the mother's titre exceeded the infant's titre by a single-fold dilution and in 7 of 112 pairs by a twofold dilution. Finally, in 12 of the 112 pairs, no EBV antibody could be detected. No permanent cell line could be obtained from the cord blood of these newborns except in one case in which half of the cord blood was purposely infected with EBV in the laboratory[13] and yielded a permanent cell line.

The sera of another group of 114 infants admitted to Sainte-Justine Hospital from birth to age 3 years were screened for EBV antibodies (Table II). Seventy-two were EBV-negative and 42 were EBV-positive. Seroconversion in the absence of apparent illness or associated with poorly differentiated respiratory and enteric syndromes occurred in 6 (10%) of the 60 EBV-negative children from whom two or more serum samples were available over a period of 18 months following their hospital admission. Five of the six, however, converted within the first six months. In none of these cases did the Monospot test become positive. The prospective follow-up of these converters and their families (only 15 of 109 family members were EBV-negative) has failed to uncover cases of infectious mononucleosis to date. Among the 42 EBV-positive infants was a 3-year-old child admitted with pharyngitis and a rash, who subsequently developed characteristic heterophil agglutinins. She had an EBV antibody titre of

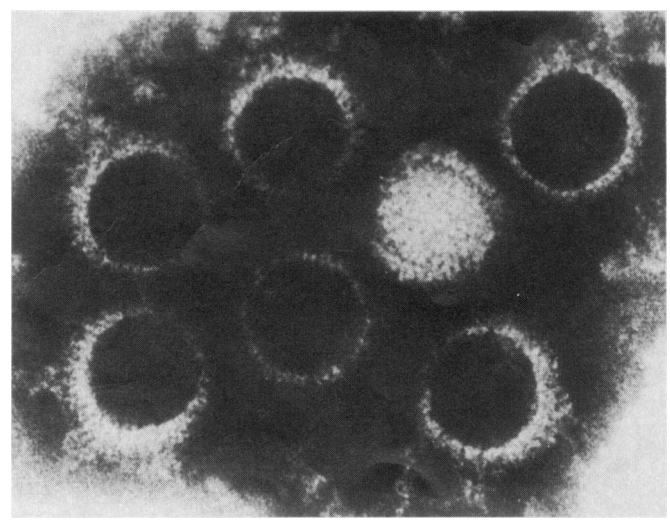

FIG. 1—Herpesvirus particles found in the high-speed pellet of a sample of the lymphoblastoid cell line established from the peripheral leukocytes of case Ia following exchange transfusion.

*Viral Genetics Branch, National Institutes of Health, Bethesda, Maryland 20014

Table I—Leukocyte culture pre- and post-exchange transfusion in newborns: EBV antibody titre at birth and at follow-up

Case	EBV antibody titre*								Leukocyte culture	
	At birth		At follow-up						Duration of metabolic activity and active "clump proliferation"	
			Age in months							
	Pre transfusion	Post transfusion	3	6	9	12-15	16-20	21-24	Pre transfusion	Post transfusion
Pa	1/5	—	—	1/5	1/5 >1/8**	—	1/5	1/20	2 weeks	—
Du	0	0	—	—	—	—	0 <1/4**	0	ct	—
La	1/40	—	0	0	—	0	1/10		2 weeks	—
Ta	1/80	—	—	—	—	—	—		2 weeks	—
Ho	0	0	—	—	—	—	0 ±1/4**	0	6 days	—
Cl	1/40	—	—	—	—	—	0 >1/8**	1/10	6 days	—
Bo	1/40	—	—	0	0	—	0	0	6 days	—
Fl	1/40	—	0	0 1/4**	—	—	—	0	6 days	—
Si	1/20	—	0		0	0	0 1/4**	0	ct	—
Pe	1/80	1/40	0	0	0	0	0		2 weeks	3 weeks
Ba	1/10	1/40	—	—	—	—	—		1 week	—
Bel 1	1/80	1/40	—	—	—	—	—	0	2 weeks	3 weeks
Bel 2	1/80	1/40	—	—	—	—	—	0	2 weeks	3 weeks
Gaud	1/20	1/40	0	0	0	0 1/8**	0 0		2 weeks	
Lem	—	1/160	—	—	—	—	0		—	3 weeks
Ia (female)	1/20	1/40	0	—	0 1/8**	—	—	1/20	3 weeks	permanent
Ia M Donor 1 (male) Donor 2 (male)	1/40 1/160 1/40								6 weeks	 6 weeks
Ro	1/10	1/10	0	0	0		—		2 weeks	3 weeks
Va	1/20	—	1/5 1/8**	—	—	—	—		2 weeks	3 weeks
Lep	1/80	1/40	—	0 1/4**	—	0	0		3 weeks	permanent
Bl	1/40	1/60	—	—	1/40	1/5 1/8**	1/10		—	6 weeks
Bl M	1/40								—	
Gaut	1/20	—	—	—	—	0	—		2 weeks	ct
Gaut M	1/20								4 weeks	
Sa	—	—	—	—	—	—	—		1 week	—
Ry	1/40	—	—	—	—	1/80	—		2 weeks	2 weeks
Mon	1/40	—	—	—	—	—	—		—	3 weeks
Mon M	1/160								4 weeks	
Mor	1/40	1/80	—	—	—	—	0		2 weeks	3 weeks

*Indirect immunofluorescence except when indicated otherwise
**Complement fixation: courtesy of Dr. Paul Gerber, Viral Genetics Branch, National Institutes of Health, Bethesda, Maryland
ct = Contaminated
— Not done parents: refused
M = Mother
0 = < 1/5

1/20 on admission and 1/160 three months later, rising eventually to 1/320 with no other illness during this period. Significant antibody fall was noted in several children and, more interestingly, definite seroreversion was observed in three children over a period as short as 6 to 17 months. These results were confirmed in duplicate experiments. Passive transfer of antibodies by transfusion or other blood derivatives was definitely ruled out in these three children who were over 1 year of age. Two of the three are still EBV-negative after one year. One of the three was EBV-positive at a titre of 1/160 when first tested, became EBV-negative six months later but positive again at a titre of 1/80 after another six months.

Discussion

Permanent lymphoblastoid cell lines could not be established from blood samples taken prior to exchange transfusions. This appears to be the rule since cord blood leukocytes if not superinfected with the EB virus or leukocyte transforming agent (LTA) have never given rise to permanent cell lines in other laboratories to date.[13-16] Permanent cell lines were obtained, however, from two newborns following transfusion. Although both infants became EBV-negative by immunofluorescence at the age of 3 or 6 months the possibility of an abortive infection without antibody response has not been discarded in view of the female karyotype of the lymphoblastoid cell line obtained in one (Ia). This possibility is reinforced by the finding of EBV antibodies by complement fixation test in both cases at ages 6 and 9 months. The possibility that the infection of these two newborns' leukocytes occurred *in vitro* from contact in mixed culture with the degenerating donor leukocytes following removal of the antibody-rich plasma has to be considered. While these babies were and are still EBV-negative by immunofluorescence we have once, without success, and will again attempt to obtain lymphoblastoid cell lines from their leukocytes. We will also examine their leukocyte DNA by molecular hybridization with EBV complementary RNA for the presence of EBV-DNA sequences.[15] Persistence of EBV antibodies throughout the first year of life in two newborns (Pa and possibly Bl) may represent instances of congenital infections. Sera from these babies will be tested for EBV IgM antibodies as soon as this technique[17-21] becomes more easily reproducible in our own and other laboratories.

The determination of EBV antibodies in sera from mother and infant at birth confirmed the results of a similar study done in Japan.[22] The finding of significantly higher newborn than maternal EBV antibody titres in three cases was also suggestive of abortive congenital infections.

The EBV conversion rate of 10% (6 of 60) among the EBV-negative children aged 0 to 3 years was quite similar to that found previously in contacts of cases of mononucleosis.[6] The conversion rate in our experience, with or without known index cases of infectious mononucleosis as a source of infection, was therefore not significantly different. Similar results with no conclusive evidence to the contrary have been reported by others.[1-9] Clinically the EBV infection in the neonatal period and in childhood, with one exception, appeared to be largely inapparent from the results of this study. Similar observations have been made by others.[2,23] The EBV infection in these age groups did not appear to be more readily transmitted to older children and young adults from the same family but the number of susceptibles was very small.

Seroreversion from positive to negative, observed in three children in this study, has not been reported previously in adults. The prospective study done in sera from children in the Cleveland family study[2] has shown a transient reversion in one case similar to that seen in one of our three cases. Excluding the immediate postnatal period, characterized by the disappearance of maternal antibodies, a two-peak curve has been noted in the rate of seroconversion and in the prevalence of the EBV antibodies in relation to age. The lowest point of the curve was found not in the younger but in the older age group, 5 to 10 years in at least two surveys,[2,6] further suggesting the possibility of seroreversion on a group basis. Careful follow-up of the EBV-positive children of the present study should determine how frequently back-conversion from positive to negative does occur and if these individuals ever develop infectious mononucleosis at a later age.

Addendum

Molecular hybridization tests kindly performed by Dr. H. Zur Hausen on the leukocyte DNA of patients Ia, Lep, Bel 1 and 2 at age 2 years were negative. Three of these patients were still EBV-negative while Baby Ia was then EBV-positive.

Leukocyte cultures done in follow-up after the age of 1½ years in nine of the 25 babies yielded a permanent cell line in three of four EBV-positive babies (Pa, Cl, Ry) but in none of five EBV-negative babies (Bo, Si, Gaut, Gaud, Lem).

The collaboration of the neonatology section at Sainte-Justine Hospital (Drs. Bernard Doray and Harry Bard) is gratefully acknowledged. Sincere thanks are extended to Dr. Louis Dallaire, Sainte-Justine Hospital, for the karyotype analysis of the established cell lines, to Drs. Maria Del Valle Pison, Maurice Fournier and Pierre Turgeon for their help in the follow-up of the children.

Table II—EBV infection in infancy and early childhood

No. of cases (total: 114)	No. of cases	Intersampling period	Titre
EBV-negative: 72			
— Two or more serum samples: 60	With seroconversion: 6/60	<4½ mos. (1 case) <5 mos. (2 cases) <6½ mos. (3 cases)	<5-40 <5-160 <5-40 <5-20 <5-80 <5-80
— One serum sample only: 12			
EBV-positive: 42			
— Two or more serum samples: 22	With significant antibody rise (with heterophil-pos. IM): 1		20-160-320
	With significant antibody fall: 5		
	With seroreversion: 3	17 mos. 5 mos. 5½ mos.	10- < 5 160- < 5 80- < 5
	Without change in titre: 13		
— One serum sample only: 20			

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Retrospect

Tonsillar infection and systemic disease

The works of Billings and others on focal infection marked a new era in medical knowledge. The suggestive theories of ten years ago have become accepted truths to-day and along these lines perhaps no region has attracted more attention and investigation than that of the tonsils.

The importance of the tonsils as portals of infection or primary foci in certain cases of systemic disease is now established beyond any reasonable debate. We have all seen troublesome cases of arthritis and "muscular rheumatism" clear up in a striking manner after a tonsillectomy. In other cases when the association of the disease with tonsillar infection has seemed clear the results of operation have not been as good as we had hoped for.

No reasonable man would suggest that the tonsils are the primary focus in all cases of arthritis, nephritis or the many other diseases where a focal infection seems likely to have been the starting point, but it has certainly been established that they frequently are at fault and should be carefully considered and investigated in all such cases.

Possibly the tonsil has received an undue share of blame and attention from the fact that it lends itself to the method of elimination in the search for all possible foci of infection. With the public demand for efficiency and action on the part of the medical man the tonsil is possibly sometimes needlessly sacrificed in grasping at a forlorn hope in the treatment of some refractory disease. Editorial: Can Med Assoc J *14: 52, 1924.*